The Isolation of Crystalline L-5-Vinyl-2-thio-oxazolidone from the Seeds of Cruciferous Plants (*Cruciferae*)

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Astwood and his colleagues[1] presented a method for the isolation of L-5-vinyl-2-thio-oxazolidone (VTO) from the seeds of cruciferous plants and from the roots of rutabaga. The losses are, however, great when this method is used to isolate pure VTO. A method has been developed in this laboratory which gives a good yield of VTO of high purity. The isolation of VTO from seeds of winter rape by the new method is described below.

Enzymatic formation of VTO from the precursor-glucoside (progoitrin) and extraction of the VTO formed. Two hundred grams of winter rape (*Matador*) seeds are crushed in either a mortar or a hammer mill, 700 ml of water is added and the mixture is covered and left standing overnight (the optimum standing time at room temperature is 12 h). After this the mixture is heated above 60°C for about 15 minutes and the seed meal is separated from the liquid with gauze. The solution is filtered and the combined seed meal and residues are washed with hot water until the total volume of filtrate and wash water is 1—1.5 l. The extract is now concentrated *in vacuo* to 400—500 ml and shaken three times with an equal volume of ethyl acetate. To avoid the formation of an emulsion, the shaking must not be too vigorous, especially at the beginning. The ethyl acetate is evaporated *in vacuo* with gentle warming at a temperature not exceeding 60°C. The residue is washed with chloroform into a 100 ml flask and the solvent is evaporated *in vacuo*.

To remove ethyl acetate completely, chloroform is again added to the residue and the vacuum evaporation is repeated. This chloroform treatment is repeated several times. The ethyl acetate-free residue is then dissolved in a small volume of chloroform. If large amounts of VTO are isolated at a time, the extracts from several 200-g batches of seed can be combined at this stage.

Purification with aluminium oxide. The chloroform extract is fractionated on an aluminium oxide column (diameter 2 cm and height 5 cm) with 100—200 ml of chloroform.

Extraction of VTO from chloroform into water and further into ethyl acetate. To free VTO further from other, mostly fat-like substances slightly soluble in water, extraction with water is performed. To achieve this the chloroform effluent is evaporated to dryness and the residue is transferred with chloroform to a Mojonnier flask of suitable size. The VTO is extracted from the chloroform phase into water with several portions of water. The transfer of VTO can be followed either by direct photometry or by a spot reaction in UV-light. Using a Mojonnier flask of 90 ml capacity, about 95 % of the VTO is removed when the extraction is repeated ten times. The yield of VTO can be increased further by using smaller solvent volumes and smaller flask sizes in the succeeding extractions. The chloroform extract is concentrated by evaporation *in vacuo* to $^1/_5$ of its original volume, for example, and the extraction is continued with smaller volumes of water.

In order to transfer VTO from the water solution to ethyl acetate, the combined water solutions are shaken with twice their volume of ethyl acetate in a separating funnel.

Purification of the crude VTO with activated carbon and the crystallization of VTO. For further purification the ethyl acetate extract is evaporated to dryness *in vacuo*, the brownish oil-like residue is dissolved in a small volume of peroxide-free ether dried with metallic sodium. A knifebladeful of activated carbon is mixed with the ether solution by gentle shaking. The carbon is separated by filtration and the ether is evaporated off. The residue is a greenish oil. It is dissolved in a small volume of methyl alcohol and the solution is placed in a cooling mixture (dry ice-methyl alcohol). A 4 l Dewar bottle, for instance, can be used. The crystalline mass is washed twice with a small amount of cold methyl alcohol. Recrystal-

lization is performed using methyl alcohol containing 10 % ether as solvent. In the next recrystallization the ether content is raised to 20 %. The ether content is further raised until a solvent containing 90 % ether has been reached. At each stage the crystalline mass is washed with cold ether after decantation of the mother liquor. The crystalline mass obtained in the last crystallization is dried *in vacuo* and is finally crystallized once more using pure ether as solvent. The yield of crystalline VTO is 70—80 % of the total amount of VTO formed in the water suspension of the seed meal.

The melting point of VTO dried in a vacuum desiccator is 50°C, but when a pressure of $3 \cdot 10^{-2}$ mm Hg and Molecular Sieve 4a absorbent are used, the melting point rises to 50.5°C. The spectrum of VTO in water shows a strong maximum with log $\varepsilon_0 = 4.23$ at 240 mμ. The mass spectrum of VTO is seen in **Fig. 1.**

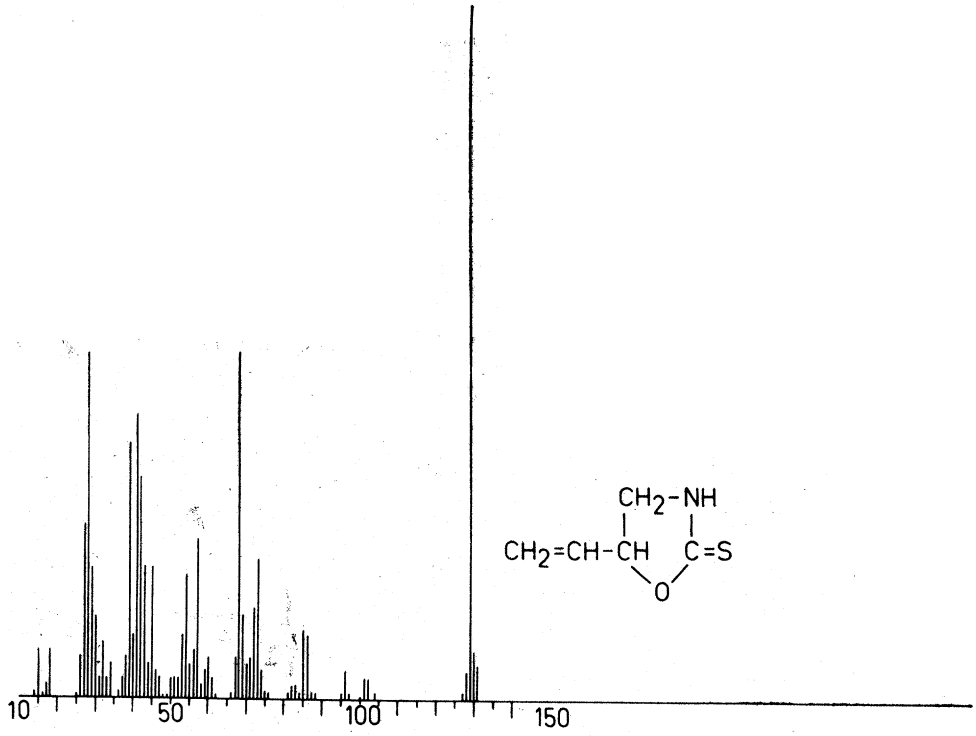

Fig. 1. The mass spectrum of L-5-vinyl-2-thiooxazolidone (VTO) from winter rape (*Matador*) seeds.

In parallel with the isolation of VTO, it is possible to dissolve the fat from the crushed seeds with petroleum ether and to extract progoitrin from the solution with 70 % methyl alcohol. Progoitrin is then split enzymatically and the liberated VTO is isolated and purified as described above.

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Reference

1. Astwood, E. B., Greer, M. A. and Ettlinger, M. G., *J. Biol. Chem.* **181** (1949) 121.

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